

Influence of Host on Behavior of *Sclerotinia sclerotiorum*

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ABSTRACT All these hosts became less susceptible as the plants matures. Ascospores were unable to infect linseed leaves in the absence of an exogenous nutrient base. Even though sucrose could be used as an exogenous nutrient to stimulate the ascospores to germinate, the ascospores subsequently failed to infect undamaged linseed leaves. Linseed flower petals were an effective nutrient base which stimulated ascospores to germinate as well as to infect undamaged linseed leaves.

Key words: Host influence, *Sclerotinia sclerotiorum*

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary causes white mold on a wide variety of morphologically different botanic organs, such as the flowers, fruits and roots of numerous taxonomically diverse hosts (Pethybridge *et al.*, 1921; Hine & Wheeler, 1970; Li & Fu, 1981; Grau, *et al.*, 1982; Boland & Hall, 1982 & 1986; Jellis *et al.*, 1984). Mitchell *et al.* (1986) suggested the need for care in selection of break crops to avoid infection by this long-lived, soil-borne pathogen.

The susceptibilities of a few crops, including soybean (*Glycine max* (L.) Merr.) (Boland and Hall, 1986; Grau *et al.*, 1982; Grau and Radke, 1984), pea (*Blanchette* and Auld, 1978), as well as linseed (*Linum usitatissimum*) (Mitchell *et al.*, 1986), have been reported before, but not in relation to the growth stages of the crops.

The successful infection of leaves by ascospores usually requires exogenous nutrients (Purdy *et al.*, 1953; McLean, 1958; Newton *et al.*, 1972; Abawi *et al.*, 1975a; Abawi *et al.*, 1975b). Sucrose increases the virulence of ascospores to sunflower leaves (Sedun & Brown, 1987). Flower petals are also believed to act as an important energy source that may support the infection of ascospores (Sutton & Deverall, 1983).

A comparative study was made of the effect of three nutrient sources on the infection of linseed by hyphae from ascospores in this study.

Willetts and Wong (1980) concluded that crops are more readily infected by some types of inocula than others. Therefore this investigation was carried out to compare the virulence of hyphae originating either from myceliogenic or carpogenic germination of sclerotia to linseed.

MATERIALS AND METHODS

Susceptibility at different growth phases

Linseed (cultivars Antares and Atalante) were grown in John Innes No.2 compost in 15 cm plastic pots in glasshouse at 20-25 °C. For each cultivar, 15 plants at each growth phase were inoculated with the mycelium of the fungus on their leaves and stems, and 6 plants of each cultivar in each growth phase were left as uninoculated controls.

Comparisons were made during three growth phases of linseed (seedling with primary leaves; late vegetative with 3-5 leaves; flowering).

Sclerotia of *S. sclerotiorum* isolated from oilseed rape plants by the ADAS at Wye, Kent were incubated at 22 °C on plates of 3.9% (w/v) potato dextrose agar (PDA) for there days in order to produce mycelium. Discs (5 mm diameter) of mycelium, cut from the advancing edge of the PDA culture were inoculated on the newest leaves and the base of the stems of each cultivar at different growth phases. When the stems were inoculated, the mycelium discs which were pressed lightly onto the bases of the stems were held in place with soil behind the discs. Immediately after inoculation, the plants were covered by plastic bags to keep the humidity as high as possible.

Disease development was observed and recorded 7 days after the inoculation. The incidences of disease, leaf death and dead plants caused by the fungus were recorded and employed as the criteria of the susceptibility of the hosts at the different growth phases. The data from the observation was analyzed by using a χ^2 test method.

Pathogenicity-comparative virulence of hyphae from established mycelia ascospores Mycelium discs prepared as described before, were inoculated at the centers of nine detached leaves of each variety. Three

leaves were treated in a similar way but with uninoculated PDA discs as controls. Ascospores were suspended in sterilized distilled water and the concentration was 2×10^4 spores/mL, then a drop (about 0.03 mL) of the ascospore suspension was placed on a flower petal on center of each detached leaf. Nine leaves were treated with ascospores, and three leaves treated with water as controls.

Detached leaves were cut from two weeks old linseed (cv. Antares and Atalante) and placed on water agar (containing 20 µg/L benzimidazole) prepared by adding 5 g "Bacto-agar" to 1000 mL distilled water and autoclaved at 121 °C for 15 minutes, 40 mg benzimidazole was added in the agar when the agar had cooled down to about 50 °C.

The disease development and incidence were observed daily after inoculation for 7 days. The data from the observation was analyzed by using a χ^2 test method.

Effects of nutrient sources on infection by ascospores Nine replicates of each of different treatments of the linseed cultivar, Antares, were grown in John Innes No. 2 compost in 15 cm plastic pots at temperatures around 20-25 °C for two weeks under glasshouse conditions. The newest leaves were then detached at same time for the different treatments.

The ascospores were prepared as described before. The same batch of ascospores was suspended in sterilized distilled water. The concentration of the ascospore water suspension was 1.5×10^4 spores per mL. The suspension was then mixed with different nutrients.

A drop (about 0.03 mL) of the ascospore suspension was placed on the center of detached leaves on water agar in plastic boxes. Nine leaves were treated with the ascospores, and three leaves treated with water acted as controls.

The ascospores were suspended in 5 M sucrose solution, and then inoculated on nine detached leaves.

Linseed flower petals from glasshouse were placed on the center of nine leaves. A drop (about 0.03 mL) of the ascospore water suspension was then placed on each petal.

All the leaves treated in these three ways were then kept at 22 °C in laboratory. Disease development was observed 8 h, 12 h, 16 h, 20 h, 24 h, 36 h, 48 h, and 168 h after inoculation.

Germination of the ascospores was observed by counting 100 ascospores under a light microscope, recording the number germinated in water, 5 M sucrose solution and water with flower petals (5 petals/mL) 12 h after the spores were put into the different nutrients. Every treatment was replicated three times. The data from the observation were analyzed by using F-test and

T-test.

RESULTS

Susceptibilities of different growth phases

Although disease incidence was similar at all the phases of growth of linseed following leaf inoculation, the highest mortality of linseed was observed at the seedling phase and appeared quite early, about two days after inoculation (Table 1). When the stems of linseed variety, Antares, were inoculated with mycelium, no difference in disease incidence could be observed between any growth phase. However, the mortalities were different at the three different growth phases (Table 2) with the highest mortality at the seedling phase and the lowest at flowering

Table 1. Response of linseed to leaf infection by *S. sclerotiorum* at three growth phases

Response of plants to the treatments	Phases of growth	Varieties of linseed	
		Antares	Atalante
Incidence of disease (%)	Seedling	100	100
	Late V	100	100
	Flowering	100	100
Mortality of plants (%)	Seedling	100Aa*	100A*
	Late V	60B	40B
	Flowering	0C	0C

Note: The results were recorded 7 days after inoculation.

a The percentage values with no letter in common differ significantly ($p < 0.001$)

* The mortalities of the two varieties as seedlings were recorded after two days of inoculation.

Table 2. Stem infection of linseed cv. Antares by *S. sclerotiorum* at three growth phases

Response of plants to the treatment	Phases of growth	Varieties of linseed	
		Antares	Atalante
Incidence of disease (%)	Seedling	100	
	Late	100	
	Flowering	100	
Mortality of plants (%)	Seedling	100 Aa	
	Late	66.7B	
	Flowering	22 C	

Note: Results were recorded seven days after inoculation.

a the percentage values with no letter in common differ significantly ($p < 0.01$).

Comparison of virulence of mycelium with ascospores Mycelium inoculated on leaves displayed very high virulence to all the crops in this experiment. The result from detached leaf test showed that (Table 3) incidence of serious leaf rot was complete on all the crop following mycelial infection. All the leaves were infected and showed symptoms of leaf rot within only two days.

Ascospores were very virulent to the crop when flower petals were a nutrient base for the spores (Table 3). All the varieties of linseed were infected.

Comparing the disease incidences caused by mycelium and ascospores on the detached leaves, the virulence seemed more or less similar. However, the disease development after ascospore infection was slower than following mycelial infection.

Effects of sources of nutrients on infection by ascospore When the ascospores of this pathogen were suspended in water they were not able to infect the leaves of the cv. Antares (Table 4). When the ascospores were inoculated in a 5 M sucrose solution, no leaves became infected (Table 4).

Ascospores of *S. sclerotiorum* readily infected leaves from the cv. Antares by first utilizing the flower petals very quickly (Table 4). Symptoms of petal rot were first observed about 8 hours after inoculation. About 16 to 48 hours after inoculation, the leaf started to rot. By about 5 to 7 days after inoculation, all the leaves showed the symptoms of leaf rot and the symptoms spread virtually all over the leaf.

Germination of ascospores in different nutrients

Ascospores were able to germinate in all the solutions, but some differences were observed (Table 4). The highest germination of ascospores was recorded with flower petals, whereas ascospores in water had the lowest germination. Although the germination of the ascospores in sucrose solution was not as high as that with petals, it was still much higher than the rate of the ascospores in water.

The statistical analysis of the data indicated (Table 4) that germination of ascospores in water was significantly less than that in the 5 M sucrose solution, but germination in the sucrose solution was significantly lower than that in the linseed flower petal washings.

Table 3. Test of the virulence of mycelium & ascospores with petals on detached leaves of the 3 crops

Crops	Varieties	Incidences (%)	
		Mycelium ^a	Ascospores ^b
Linseed	Antares	100	100
	Atalante	100	100

Note: ^a This result was recorded two days after inoculation. ^b this result was recorded 7 days after inoculation.

DISCUSSION

The comparisons of the response of linseed to the infection of mycelium of *S. sclerotiorum* at the different growth phases showed that the linseed varieties responded to the infection of mycelium of the pathogen differently in the different growth phases, all tended to be

more susceptible to mycelial infection at the seedling phase. This result is similar to that observed in soybean (Yang Qian, 1996).

Table 4. Incidence of the disease on leaves of the linseed cv. Antares by mycelium and ascospores of *S. sclerotiorum* and germination of ascospores in different nutrients

Treatments of the host crops	incidence of disease (%)	germination (%)	
		Mean	S ²
mycelium + PDA	100		
ascospores + water	0	48.0 Aa	0.19
ascospores + sucrose	0	75.3 B	0.02
ascospores + petals	100	95.3 C	0.34
			6.8*

Note: a The values with no letter in common differ significantly ($p < 0.05$, $p < 0.05$ respectively).

* S. E. D. of the three germination values (d.f. = 7).

The mode of infection tends to vary under different conditions. Ascospores are considered an important source of inoculum, especially for *S. sclerotiorum* (Willets and Wong, 1980). Attack by ascospores tends to take place when the hosts are blooming (Grau and Radke, 1984). However, the observations from this study indicated that the flowering phase is not the most susceptible stage in the life cycles of linseed. Instead, the environmental and pathogenic factors seem more important (Grau and Radke, 1984), which suggests the possibility that, like partial resistance, breeding plants for lower susceptibility at flowering could play an important role in a rational approach programme to control the disease.

The significant changes in the susceptibilities of different plants emerges at different stages in the life cycle. It is between the seedling and late vegetative phases, and between the late vegetative and flowering phases.

Although the variability of the susceptibilities in different growth phases were observed in the crop, interpretation of these reactions is not straightforward. Grainger (1956) suggested that the high susceptibility, disease potential, of potato to *Phytophthora* blight in its growth cycle was associated with high percentage of total carbohydrate in the whole plant. Chen Yongkang (1960) showed that the changes in susceptibility between growth phases were possibly linked with the nitrogen metabolism. Therefore, there is the potential to link fluctuations in disease potential with some changes in nutritional metabolism in the growth cycle of the hosts.

Comparison between mycelium on PDA and ascospores on petals showed that the virulence of the two inocula are similar, although infection by ascospores is slower than that by mycelium. Therefore, hyphae from mycelium appear to be reliable as an inoculum for

screening varietal resistance or susceptibility. This investigation also corroborate the procedures adopted in earlier work on soybean, field pea, bean and lettuce (Boland and Hall, 1986; Blanchette and Auld, 1978; Hunter *et al.*, 1981; Madjid *et al.*, 1982/83).

The results from the comparison of effects of nutrients on the infection by ascospores indicated that for successful infection of leaves by ascospores of *S. sclerotiorum*, flower petals were very important to supply energy, confirming the observation of Abawi *et al.* (1975b) on *Phaseolus* bean.

Although sucrose could stimulate and support the germination of the ascospores, it could not stimulate the infection of leaves by ascospores unlike the findings on sunflower by Sedun *et al.* (1987). The discrepancy between the effects of flower petals and sucrose demonstrates that the flower petals do not only act as energy source for ascospore germination but also stimulate the infection process. As ascospores were unable to infect leaves without a nutrient base, this confirmed most previous investigations but conflicted with the observation on soybean by Sutton *et al.* (1983). Therefore, ascospores may behave differently on different crops under different conditions.

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(Responsible: Editor: Sun Yueqi).